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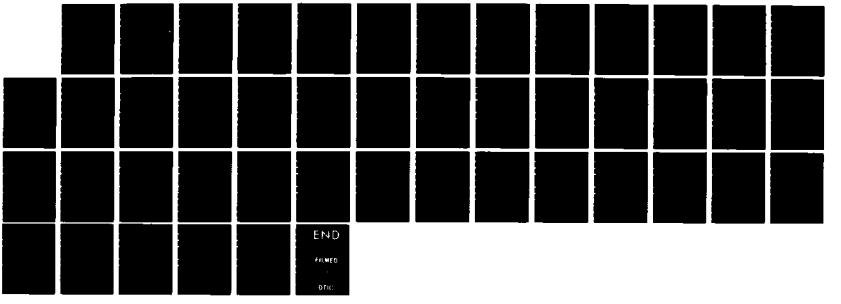

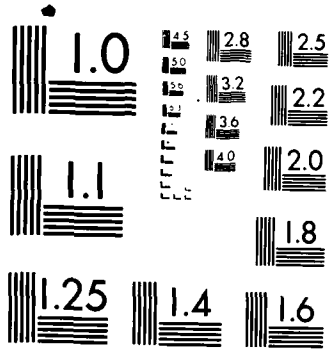

CHEMOTHERAPY OF LEISHMANIASIS

Annual Report

by

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September 1979

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Additional isolates now received numbering from LV678 through LV700 are listed. The isolates include important visceral strains from Honduras, Italy, France and India. The majority of other isolates were sent for identification from various laboratories where they are being used in current investigations. The most interesting findings this year include the identification by Dr. Chance of Leishmania isolated by Professor Bettini from dogs, Rattus rattus and a fox in Italy as L. donovani s.l., of the same enzyme type as visceral isolates from man in the Mediterranean region. This is the only recent clear incrimination of

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BLOCK 20 CONT'D

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INTRODUCTION

A Final Report relating to work carried out with support from the previous Grant No. DAMD17-77-G-9435 was submitted in December 1978. The present Report covers further data acquired under Grant No. DAMD17-79-G-9456 from January through September 1979. At this time the Principal Investigator is transferring his activities in the field of leishmaniasis chemotherapy research to London where he will head the Department of Medical Protozoology at the London School of Hygiene and Tropical Medicine from October 1 1979. The present Report follows the format recommended in a letter from WRAIR SGRD-AJ dated October 11 1978.

SCIENTIFIC ACTIVITIES

1. CHEMOTHERAPY

1.1 Techniques

The techniques developed or adopted in this laboratory for the study in vivo of the action of potential antileishmanial drugs against cutaneous or visceral infection have now been described in detail in a series of 4 papers submitted for publication (** p.7) of which advance manuscript copies have been forwarded to WRAIR for study. These papers included a summary and analysis of many compounds in a broad spectrum of chemical classes, and suggestions for further investigations in this field. For convenience the discussion section of the final paper is included here as Appendix 1.

Technical details of the procedures followed here in mouse models to test for activity against Leishmania major, L. mexicana amazonensis and L. donovani sensu lato were given in the last Final Report and are expanded on in the papers to be published. We have as yet not succeeded in establishing a reliable mouse model for L. panamensis or L. braziliensis sensu stricto. However further in vitro tests were carried out by the technique described earlier by Mattock and Peters (1975)* using tissue cultures and data are provided below.

1.2 Data on WRAIR and other compounds tested in vivo

In Table 1 are summarised data obtained with 13 compounds supplied by WRAIR. Details are provided in Tables 2 through 15.

The significant findings in L. donovani infected mice may be summarised as follows:-

(i) Glucantime had an ED_{90} po of about 360 mg/kg as compared with 200 mg/kg sc.

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* Mattock and Peters (1975). Ann. trop. Med. Parasit., 69, 349-357.

(ii) 4-methyl primaquine had an ED_{90} of $\sim$ 13 mg/kg po as compared with 10-12 mg/kg sc.

(iii) 2-methyl primaquine was highly active po with the ED_{90} $<$10 mg/kg, but showed poor activity sc (ED_{90} $>$ 100 mg/kg).

(iv) WR 211666 gave an ED_{90} po of $\sim$ 9 mg/kg.

(v) Mefloquine showed no significant action po or sc

(vi) WR 225448, and 5990 had an ED_{90} of $>$ 30 mg/kg sc (i.e. the screening dose).

(vii) WR 227495 had an ED_{90} of $<$ 30 mg/kg

(viii) WR 221527 and 219423 had ED_{90} of $<$10 mg/kg

Against L. major

(ix) Mefloquine was inactive, WR 113618 had an ED_{90} of 94 mg/kg sc, WR 135403 205 mg/kg sc and 2-methylprimaquine 135 mg/kg sc.

(x) BH 73074 was inactive against L. m. amazonensis sc.

Unfortunately all recent tests against cutaneous parasites produced poor control infections and will have to be repeated after the move to London has been completed.

1.3 Drug activities in tissue culture

The data summarised in Table 16 were obtained by Dr. Mattock who was able to rejoin the Department for a short time on a temporary basis. The technique employed was that of Mattock and Peters (1975, loc.cit.) using either mouse peritoneal macrophages or dog sarcoma cells, and the same lines of parasite as used in WRAIR in vivo (see Table 16).

These data provide an interesting comparison with those obtained in the in vivo mouse serum.

(i) Amphotericin B shows good activity in mouse peritoneal macrophages (MPM) infected with L. donovani s.l. and L. m. amazonensis, and slightly less activity against L. panamensis and L. major. It is only active at a high dose in vivo against the two parasites against which it has been tested, i.e. L. donovani s.l. and L. major.

(ii) Nystatin is highly active against L. panamensis and L. m. amazonensis in MPM and a little less active against L. donovani s.l.

(iii) 2-methyl and 4-methyl primaquine are highly active against all four species in MPM with slight variation between species. In vivo 4-methylprimaquine was much more active against L.donovani s.l. than against L. major or L. m. amazonensis sc or po. 2-methyl primaquine has been tested so far against L. donovani against which it is highly active po (but not sc), and L. major in which it has a low level of activity sc. (It has not yet been checked po).

(iv) WR 6026 and WR 211666, both of which were highly active in vivo against L. donovani s.l. sc and po, but not against L. major or L. m. amazonensis, show little or no action against any parasite in MPM, which would suggest that they may undergo a metabolic transformation to active derivatives in the liver. However both compounds were active against L. m. amazonensis in DS.

(v) No action was obtained with allopurinol, oxypurinol, 25-hydroxycholesterol or BH73074 in MPM. Allopurinol has some activity against L. donovani in vivo.

2. PARASITE BIOCHEMISTRY

2.1 Carbohydrate metabolism

Using the separation technique he recently described (Brazil, 1978)* Brazil has examined the carbohydrate metabolism of amastigotes of L. m. amazonensis and their nucleic acid synthesis. Starch gel electrophoresis was valuable as a means of identifying initially which enzymes were of parasite origin, and established that the amastigotes free of contaminating host cell possess glucose phosphate isomerase (GPI), glucose 6-phosphate dehydrogenase (G6PD) malate dehydrogenase (MDH) and isocitrate dehydrogenase (IDH). Quantitative studies failed to demonstrate that amastigotes catabolise glucose in vitro up to 24 hours in Ho-MEM medium. If any was used it was less than could be detected by the God-Perid method used in this study. When ^{14}C glucose was used no labelled metabolite could be detected from 1 to 24 hours confirming that amastigotes maintained in vitro do not use glucose as their main energy source. The chromatogram of the final medium after the incubation of amastigotes in Ho-MEM medium is shown in Figure 1. The only peak corresponds to that of the glucose standard.

2.2 Nucleic acid metabolism

In vitro it was shown that amastigotes of L. m. amazonensis readily incorporate ^{3}H adenosine and ^{3}H uridine, but not ^{3}H thymidine and ^{14}C orotic acid into nucleic acid (Figures 2 and 3). The non-incorporation of thymidine would suggest that amastigotes do not synthesise DNA from thymidine but possibly from uridine as suggested by autoradiographic studies in infected macrophages by Bhattacharya and Janovy (Exp. Parasit., 1975, 37, 353). This study has provided a useful baseline for the work described below on the mode of action of pentamidine. (See also comments in section 4 on incorporation of thymidine by promastigotes.)

* Brazil (1978). Ann. trop. Med. Parasit., 72, 289-291.

3. MODE OF DRUG ACTION

3.1 Pentamidine

The effect of pentamidine in concentrations between 10^{-4} and 10^{-6}M on the incorporation of ^{3}H adenosine and ^{3}H thymidine was investigated (by Dr. Croft) on promastigotes and amastigotes of L. m. amazonensis in vitro. At 10^{-5}M pentamidine caused a 30% reduction in the uptake of ^{3}H adenosine by promastigotes after 5 hours, whereas the amastigotes showed no significant change in incorporation of the label. Promastigotes of L. donovani (LV9) and L. m. mexicana (LV4) proved to be more sensitive than those of L. m. amazonensis. Amastigotes of the last parasite incubated at 26°C with pentamidine were killed by 48 hours in concentrations of 10^{-4} and 10^{-5}M, but were unaffected by 10^{-7}M, although this slowed transformation of the amastigotes to promastigotes. However at 10^{-6}M the amastigotes were not killed but failed to transform to promastigotes for up to 10 days of observation. Ultrastructural examination of pentamidine treated promastigotes showed that early damage included extensive vacuolisation in the mitochondrion-kinetoplast region and a disruption of the kinetoplast DNA.

3.2 Sodium stibogluconate

The uptake of ^{125}Sb sodium stibogluconate has been studied using liquid scintillation counting techniques with amastigotes and promastigotes of L. m. amazonensis LV78. At a drug concentration of 10^{-4}M over 24 hours a small uptake of drug can be demonstrated and the amount is only slightly reduced by washing. Even at this high drug concentration the parasites remain alive. A simple motility test shows that promastigotes continue to thrive for at least 48 hours in a concentration of 10^{-3}M. Autoradiographic and X-ray microanalytical techniques are currently being employed to determine the sites of uptake of this drug in vitro.

4. HOST PARASITE RELATIONS IN MACROPHAGES

One of the enigmas of infection with Leishmania is how the parasites survive the destructive action of macrophagesin which they develop. Earlier studies by Lewis (Lewis and Peters, 1977)* showed that the macrophage lysosomes do fuse with the parasitopharous vacuoles but that the liberated lysosomal enzymes apparently do not attack the contained amastigotes. Following up this lead Mr. Stokes has attempted to identify some of the lysosomal enzymes both within the parasites and the host cells. The first problem has been to determine the degree of infectivity of various stocks of parasites for macrophages from various genetically characterised strains of mice.

4.1 Relation of Leishmania species and stock to host strain

An in vitro study of the infectivity of various species of Leishmania promastigotes (L. mexicana mexicana, L. m. amazonensis, L. major and L. donovani) has been made using normal (unstimulated) murine macrophages from various strains of laboratar y mouse (TFW, NMRI Inbred, C_3H/mg and Balb C). The particular strain of L. donovani used was found to have poor infectivity in all strains of murine macophages with the ratio of promastigotes to macrophages used in this study (2:1), although if amastigotes replaced the promastigotes infection was good. L. major was

* Lewis and Peters (1977). Ann. trop. Med. Parasit., 71, 295-310

reasonably infective in all the macrophage strains used ranging between 25% and 45% infection of macrophages on the 6th day after infection. L. m. mexicana and L. m. amazonensis both gave good infection; on day 6 there were infection rates of 70%-100% and 60%-90% respectively. The variation in infection rates was due to differences between macrophage strains. No one strain of murine macrophage seemed to be better with all the Leishmania species used but Balb C and NMRI macrophages were consistently good. An in vivo study of the infectivity of L. major and L. m. mexicana in the four mouse strains listed above showed up a difference in the susceptibility to infection between macrophages in vivo and in vitro. Although Balb C mice were again infected consistently well, NMRI mice were very poor. C_3H and TFW, which had not given very good results in vitro had the infection taken well in vivo. This shows that the in vitro system is not a completely true representation of the in vivo situation and care must be taken in interpretation of in vitro results.

4.2 Morphology of parasites in culture

During infection studies it was found that L. m. mexicana has a short non motile form in the overlay of infected macrophage cultures whereas the other species used had only elongate motile promastigotes present. Studies on the morphology using electron microscopy indicate this form to be amastigote-like. The incorporation of ^3H thymidine by the short form was measured and it was found not to incorporate thymidine whereas promastigotes of the same species do incorporate it. When transferred to NNN blood slopes at 26°C 100% of the short form transformed into fully motile elongate promastigotes within 2 days. This evidence suggests that at the temperature that infected macrophages are incubated (32°C) L. m. mexicana parasites released from ruptured macrophages do not transform to promastigotes whereas other species of Leishmania studied transform to promastigotes in the same situation. As the numbers of amastigote like bodies present in the overlay are quite substantial (up to 10^7 per ml), this could be a convenient method of obtaining clean amastigotes in large numbers for biochemical or chemotherapy studies.

4.3 Attempts to quantify host and parasite enzymes

Enzyme assays or whole homogenates of infected and non-infected macrophages during the course of infection have shown an increase in the activity of acid phosphatase (measured as μg nitrophenol released per hour per 100 μg protein) as the infection progresses. When heat killed parasites were taken up by macrophages the activity of acid phosphatase was lower than in infected macrophages. An attempt to assay other enzymes failed due to insufficient quantities of these enzymes being present in the homogenate.

These results became a little clearer when histochemical staining was used. Staining for acid phosphatase using Naphthol-AS-BI-phosphate coupled with Fast Dark Blue-R showed that lysosomes increase in size and numbers in infected macrophages when compared to normal macrophages, and that amastigotes have substantial amounts of acid phosphatase. In comparison macrophages with intracellular dead parasites have fewer lysosomes and the parasites have little acid phosphatase. It is hoped that histochemical staining for other enzymes will be more successful than assay techniques.

5. BIOCHEMICAL CHARACTERISATION OF LEISHMANIAL ISOLATES

Additional isolates now received numbering from LV678 through LV700 are listed in Appendix II. The isolates include important visceral strains from Honduras, Italy, France and India. The majority of other isolates were sent for identification from various laboratories where they are being used in current investigations.

The most interesting findings this year include the identification by Dr. Chance of Leishmania isolated by Professor Bettini from dogs, Rattus rattus and a fox in Italy as L. donovani s.l., of the same enzyme type as visceral isolates from man in the Mediterranean region. This is the only recent clear incrimination of rodents as reservoirs of human visceral disease, although they are, of course, commonly associated with zoonotic L. major. Further isolates that have been brought from India should help to resolve the enigma of the origin and specific identity of the organisms responsible for the current epidemic of kala-azar in that country.

Further papers are in preparation summarising our data on visceral isolates, while extensive investigations are now being made on our collections of New World isolates other than the viscerotropic parasites. This material includes a large collection from man, dogs and donkeys recently brought from Venezuela. (The latter are not yet included in the list in Appendix II which brings the total in our collection to 700).

6. PUBLICATIONS

6.1 Papers published since the last Final Report (December 1978)

Brazil, R. P. (1979). In vitro susceptibility of mouse peritoneal macrophages to Leishmania spp. Trans. R. Soc. trop. Med. Hyg., 73, 101-102.

Brazil, R. P. and McCarthy, J. D. (1979). Purine and pyrimidine synthesis in promastigotes of Leishmania mexicana amazonensis. Trans. R. Soc. trop. Med. Hyg., 73, 323.

Chance M. L. (1979). The identification of Leishmania. In: "Problems in the Identification of Parasites and Their Vectors", (A. E. R Taylor R. Muller, eds.), Blackwell Scientific Publications: Oxford, pp. 55-74.

Chance, M. L. and Peters, W. (1977). The characterisation and significance of DNA and enzyme variation in the genus Leishmania. Proc. International Congress of Protozoology held in New York, June 1977, p. 419.

Chance, M. L., New, R. R. C. , Thomas, S. C. and Heath, S. (1979). The treatment of visceral leishmaniasis with liposomes. Trans. R. Soc. Trop. Med. Hyg., 73, 321-322.

Croft, S. L. (1979). Ultrastructural study of the nucleus of Leishmania hertigi. Protistologica, 15, 103-110.

Croft, S. L. and Molyneux, D. H. (1979). Studies on the ultrastructure, virus-like particles and infectivity of Leishmania hertigi. Ann. trop. Med. Parasit., 73, 213-226

Croft, S. L., Chance, M. L. and Gardener, P. J. (1979). Ultrastructural and biochemical characterisation of strains of Endotrypanum. Trans. R. Soc. trop. Med. Hyg., 73, 322.

6.2 Papers submitted for publication

Brazil, R. P. (1979). Incorporation of precursors in nucleic acid of amastigotes of Leishmania m. amazonensis. J. Protozool.

Croft, S. L. (1979). Autoradiographic and cytochemical study of the nucleus of Leishmania hertigi. J. Protozool.

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Croft, S. L. and Molyneux, D. H. (1979). Further studies of the virus-like particles of Leishmania hertigi. J. Protozool.

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Dedet, J. P., Derouin, F. Hubert, B., Schnur, L. F. and Chance, M. L. (1979). Isolation of Leishmania major from Mastomys erythroleucus and Tatera gambiana in Senegal (West Africa). Ann. trop. Med. Parasit.,

** Peters, W., Trotter, E. R. and Robinson, B. L. (1980). The experimental chemotherapy of leishmaniasis, V: the activity of potential leishmanicides against "L. infantum LV9". Ann. trop. Med. Parasit.

** Peters, W., Trotter, E. R. and Robinson, B. L (1980). The experimental chemotherapy of leishmaniasis, VII: drug responses of L. major and L. mexicana amazonensis, with an analysis of promising chemical leads to new antileishmanial agents. Ann. trop. Med. Parasit.

Rassam, M. B. Al-Mudhaffar, S. A. and Chance, M. L. (1979). Isoenzyme characterisation of Leishmania species from Iraq. Ann. trop. Med. Parasit.

Sells, P. G. and Burton, M. (1979). The micro-ELISA test in serological diagnosis of cutaneous and visceral leishmaniasis. Parasitology.

** Trotter, E. R., Peters, W. and Robinson, B. L. (1980). The experimental chemotherapy of leishmaniasis IV: the development of a rodent model for visceral infection. Ann. trop. Med. Parasit.

** Trotter, E. R., Peters, W. and Robinson, B. L. (1980). The experimental chemotherapy of leishmaniasis, VI: the development of rodent models for cutaneous infection. Ann. trop. Med. Parasit.

APPENDIX I

Discussion from Peters, W., Trotter, E. R. and Robinson, B. L. (1980) The experimental chemotherapy of leishmaniasis, VII. drug responses of L. major and L. mexicana amazonensis, with an analysis of promising chemical leads to new antileishmanial agents. Ann. trop. Med. Parasit. (in press)

DISCUSSION

We have selected for further comparison those compounds that have exhibited the greatest activity in vivo against any of the three species examined in our laboratory ie. "L. infantum LV9" (Trotter et al., 1979, Peters et al., 1979), L. major LV39 and L. m. amazonensis LV78 (Trotter et al., 1979b and the present paper). The compounds have been divided into 6 groups. Group A contains 9 that are known to act as dihydrofolate reductase inhibitors against other organisms, 5 of them being diaminoquinazolines. It is interesting to observe that they are not necessarily those substances that show the greatest action against, for example, malaria parasites in mice. Trimethoprim, for example, is poorly active against P. berghei, whereas pyrimethamine is very active but does not figure in Table 5. Nor does WR 158122 which is also very active against rodent malaria. In its place however are several analogues less active against malaria. Neal (1972) too observed that trimethoprim was superior to pyrimethamine against L. major LV39 in mice and (Neal, 1976) also found pyrimethamine to be inactive against a line of L. mexicana.

Included also in Group A is Berenil which we find highly active, in contrast to pentamidine which is not. The mode of action of the diamidines

is uncertain.

Group B consists of 11 of the 8-aminoquinoline group including 6 lepidines (WR 203608, 6026, 211666, 212579, 226257 and 226292). Note that lepidines tested by Peters et al. (1979) and Kinnamon et al. (1978) have proved to be among the most active leishmanicides yet found. The two 6-aminoquinolines in this group are highly toxic.

Group C contains a variety of potent antimalarial blood schizontocides and is notable for not including chloroquine or quinine. Included in this group is T 1238, an amidineurea related to the antimalarial, nitroguanil.

Group D consists of a number of structures that possess trypanocidal action (nifurtimox, benznidazole), two metronidazole analogues (LIV/1319 and 1320), and two compounds with activity against schistosomes (Ro 11-0761 and Ro 10-7062). Dehydroemetine may also be included in this group as, indeed, could Berenil which is also trypanocidal.

Group E contains two antibiotics of the clindamycin group, and amphotericin B.

The final group F contains organic metallic compounds, namely Pentostam and a tin compound. It would also contain certain other organic antimonials that have not been examined yet in all these models.

Comparing the data from the above groups of compounds certain relationships between these groups and the target species are apparent. Group A compounds appear to be particularly active against L. major but relatively poor against the visceral parasite or (in the few cases examined) L. m. amazonensis. The

8-aminoquinolines are obviously more active against "L. infantum LV9" than against L. major. So far they have not been examined in mice infected with the third species. At least two compounds are highly active not only against the visceral parasite, but also against L. major WR 182234, 2-methyl primaquine is of course not a lepidine, whereas WR 226292 which is very active against all three species, is a lepidine. The two 6-aminoquinolines too are most active against "L. infantum LV9". On the contrary, Group C compounds in general are more active against the dermatropic organisms than the viscerotropic parasite, showing no or little activity against "L. infantum LV9" Group D compounds and Pentostam are equally effective against "L. infantum LV9" or L. major. Note however that the SD_{90} of Pentostam is quite different in the three species, namely 46.5,825 and 258 mg/kg sc x 5 (as Sb^V) respectively for "L. infantum LV9", L. major and L. m. amazonensis. The activity of the antibiotics against the different species is variable, and the apparent inactivity of amphotericin B against L. m. amazonensis in particular remains to be verified.

In an earlier paper (Mattock and Peters, 1975c) we suggested on the basis of tissue culture studies that the response to different compounds with modes of action known from other types of infection could give a clue to various aspects of the metabolism of Leishmania. Following confirmation of the activity of some of these compounds in vivo and inactivity of others it would now seem that the following features should be investigated:-

1. Pyrimidine metabolism. Leishmania appear not to incorporate p-aminobenzoic acid since sulphonamides and sulphones are essentially inactive. They clearly do convert dihydrofolate to tetrahydrofolate since this step is significantly blocked by certain dihydrofolate reductase inhibitors.

2. Nitroreductase-linked pathways. Metronidazole has been shown to exert a trichomonicidal action after being reduced to a hydroxylamine by a nitroreductase specific to the parasites (Coombs, 1976). Studies of this action have indicated that Trichomonas probably possesses a ferredoxin or flavodoxin which is characteristic of anaerobic organisms (Muller et al., 1976) but whether such compounds exist in Leishmania has not yet been explored. Nitroimidazoles exert their toxic action on T. vaginalis through the reduction product by a so-far undetermined interaction with cellular metabolic processes. It seems likely that other nitro-compounds possess a similar mode of action, and it is striking that several different classes of these are good leishmanicides in our models.

3. While the mode of action of 8-aminoquinolines is not yet understood it has been suggested that they interfere with mitochondrial respiration, possibly through interaction with ubiquinones. Their high level of activity against Leishmania merits investigation of the ubiquinones and cytochrome systems of these parasites which appear to offer a valuable point for selective drug toxicity.

4. The marked activity of a variety of antimalarial schizontocides is interesting in that several of them are active against chloroquine-resistant Plasmodium and have a quinine-like action. Drugs such as mefloquine do not appear to depend on interaction with plasmodial haemozoin (as does chloroquine, for example) but to have a different type of action, possibly on lysosomal enzymes or membranes. A possible interaction between these compounds and Leishmania surface membranes should be investigated as these probably play an important role in enabling the amastigotes to survive inside their host cells. It has been postulated that Pentostam too may act in a similar manner.

There appears to be a good correlation between the activity of compounds as exhibited in tissue culture and their action *in vivo* in so far as this has been tested. We have not yet however examined *in vivo* a sufficient number of compounds that proved to be inactive in tissue culture to be dogmatic on this point. Nevertheless our data, and those of Neal (summarised in Table III of Mattock and Peters, 1975b) do seem to indicate a good qualitative and even quantitative parallel between the tissue culture and *in vivo* findings in the organisms that we have so far examined. Method B gives more valuable data for both "*L. infantum* LV9" and *L. major* and *L. m. amazonensis* in the mouse, and is no more time consuming than Method A in each case. While NMRI mice were used for the visceral infection studies, other mouse lines such as BALB/c could equally well be used if they are available. TFW mice are probably satisfactory for many cutaneous organisms and are readily available, but other random-bred lines would probably serve equally well.

A close parallel exists between our animal data for the cutaneous parasites, and clinical observation insofar as the clinicians have been able to make a certain identification of the infecting organisms. This is relatively simple in areas where *L. major* is the dominant organism, or *L. infantum*, but the situation is particularly complicated in the New World where an abundance of different *Leishmania* exist of the *L. mexicana* and *L. braziliensis* complexes. Before clinical trials are made with new compounds and exaggerated claims are made for their success it is essential that every attempt should be made to isolate in culture or in laboratory animals the causative organisms so that they can be typed by modern biochemical methods. In this way the self-healing *mexicana*-induced ulcer can be differentiated from the recalcitrant lesion of, for example, Pian Bois, and the influence of any treatment on the rate of healing can properly evaluated by the clinician.

From the data we have obtained (Trotter et al., 1978a;b; Peters et al., 1979) it would appear justified to pursue further in clinical trial certain compounds that are already in clinical use, albeit it for other conditions. These include trimethoprim (against L. major), cycloguanil (possibly its progenitor, proguanil) against visceral infection (here the repository formulation of cycloguanil embonate could be useful), Berenil, mefloquine, nifurtimox, benznidazole and clindamycin (against cutaneous infections). Further preclinical development would also be justified with some of the highly potent diaminoquinazolines, with WR 113618, and possibly with di-n-octyl-tin maleate (against cutaneous infections), with lepidines against all types of infection, and notably WR 6026, WR 226292 and WR 182234. It is interesting that Kinnamon et al., (1978) and Hanson et al.,(1977), both working with a visceral infection in hamsters, have reached similar conclusions.

Two further types of compound should be included here for completeness. In Part V of this series (Peters et al., 1979) we drew attention to the activity of allopurinol and oxypurinol against "L. infantum LV9" in vivo. This important confirmation in mice of the observations of Marr and Berens (1977) on the action of these adenine antagonists against "L. donovani, L. mexicana and L. braziliensis" (as they termed them) promastigotes, and amastigotes of the visceral organism in vitro, indicate that allopurinol which is used clinicallly for its inhibitory effect on zanthine oxidase,(it is metabolised to oxypurinol in man), should be tested in suitable patients with leishmanial infection.

The second important development in leishmanial chemotherapy is the use of pentavalent antimonials incorporated in liposomes for the treatment of visceral infection in experimental animals (Black et al., 1977 New et al., 1978; Alving et al., 1978 a, b). The greatly enhanced action of these compounds

in liposomes should be followed up in larger animals and, if justified by further studies (including preclinical toxicity and tolerability studies), certainly merit clinical trial in patients with kala-azar.

APPENDIX II

Key to donors continued

49	Dr. L. F. Schnur, Hebrew University-Hadassah Medical School, Jerusalem, Israel
50	Dr. D. Le Ray, Prince Leopold Institut de Medecine Tropicale, Antwerp, Belgium
51	Professor A. B. Chowdhury, Calcutta School of Tropical Medicine, India
52	Dr. J. P. Farrell, Rutgers State University, New Jersey, USA
53	Dr. L. Hendricks, Walter Reed Army Institute of Research, Washington DC, USA
54	Dr. R. Behin, WHO Immunology Centre, Lausanne, Switzerland
55	Dr. Sergio Bettini. Instituto Superiore di Sanita, Rome, Italy
56	Dr. P. Desjeux, Institut Pasteur, Dakar, Senegal
57	Dr. R. Custodio, Tegucigalpa, Honduras
58	Dr. M. Reguer, Institut Pasteur, Cayenne, French Guyana
59	Dr. T. K. Jha, SK Medical College, Muzaffarpur, Bihar, India
60	Dr. V. Assefi, Institut Pasteur, Teheran, Iran
61	Dr. K. P. Chang, Rockefeller University, New York

LV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV626	L. major	DK81	Dedet (40)	Senegal	Man	CL
LV627	L. major	DK83	Dedet (40)	Senegal	Man	CL
LV628	L. major	DK84	Dedet (40)	Senegal	Man	CL
LV629	L. major	DK85	Dedet (40)	Senegal	Man	CL
LV 630	L. major	DK86	Dedet (40)	Senegal	Man	CL
LV 631	L. major	DK87	Dedet (40)	Senegal	Man	CL
LV 632	L. donovani	D1	Mutinga (22)	Kenya	Dog	
LV 633	L. donovani	D2	Mutinga (22)	Kenya	Dog	
LV 634	T. roussetti	-	Hommel (26)	Gabon	Rousetus aegypticus	
LV 635	L. donovani	I	Chowdhury (51)	India	Man	KA
LV 636	L. donovani	II	Chowdhury (51)	India	Man	KA
LV 637	L. donovani	VI	Chowdhury (51)	India	Man	KA
LV 638	L. donovani	VIII	Chowdhury (51)	India	Man	KA
LV 639	L. sp.	Herrera 117	Hendricks (53)	Honduras	Man	KA
LV 640	L. sp.	142	Hendricks (53)	Honduras	Animal	V
LV 641	L. sp.	Marray 158	Hendricks (53)	Panama	Man	CL
LV 642	L. sp.	Herrera 182	Hendricks (53)	Honduras	Man	KA
LV 643	L. sp.	195	Hendricks (53)	Honduras	Animal	V
LV 644	L. sp.	Hendricks 220	Hendricks (53)		Man	CL
LV 545	L. donovani	219	Hendricks (53)	India	Man	KA
LV 646	L. sp.	234	Hendricks (53)	Spain	Dog	skin
LV 647	L. sp.	009	Walton (12)	Honduras	Man	CL
LV 648	L. b. panamensis	Murray LN158	Hendricks (53)	Panama	Man	CL
LV 549	L. donovani	Khartoum WR168	Hendricks (53)	Sudan	Man	KA
LV 550	L. donovani	WR271	Hendricks (53)	Kenya	Man	KA
LV 651	L. sp.	McGillivary WR272	Hendricks (53)	Kenya Nakuru	Man	CL
LV 652	L. sp.	Orviss WR275	Hendricks (53)		Man	CL
LV 653	L. donovani	SC/SKC	Jha (59)	India	Man	KA
LV 554	L. braziliensis		Reguer (58)	French Guyana	Man	CL
LV 555	L. tropica	1	Assefi (60)	Iran, Isfahan	Man	CL
LV 556	L. tropica	2	Assefi (60)	Iran, Ahwaz	Man	CL
LV 657	L. donovani	RBS	Behin (54)	Sudan	Man	KA
LV 658	L. tropica	D26	Abdalla (25)	Sudan	Man	CL
LV 659	L. mexicana		Brazil (24)	Brazil Maranhao	Man	DCL
LV 660	T. sp.	T51	Hommel (26)	F. Guyana	Saimiri sciureus	? T. minaseuse

LV No.	Species	Isolate No.	Donor	Where isolated	Source	Notes
LV 661	L. sp.	Volpes V61	Bettini (55)	Italy, Grossetto	Fox	
LV 662	L. donovani	Lana	Bettini (55)	Italy, Orbitello	Dog	
LV 663	L. donovani	Dora	Bettini (55)	Italy, Grossetto	Dog	
LV 664	L. donovani	Cane	Bettini (55)	Italy, Puglie	Dog	
LV 665	L. sp.	DK110	Desjeux (56)	Senegal	Mastomys	V
LV 666	L. sp.	DK115	Desjeux (56)	Senegal		
LV 667	L. sp.	L69	Rioux (14)	France	Man	KA
LV 668	L. sp.	L70	Rioux (14)	France	Man	KA
LV 669	L. sp.	L71	Rioux (14)	France	Mun	KA
LV 670	L. sp.	L72	Rioux (14)	France	Dog	V
LV 671	L. sp.	L73	Rioux (14)	France	Man	KA
LV 672	L. sp.	L76	Rioux (14)	France	Man	KA
LV 673	L. sp.	L77	Rioux (14)	Tunisia	Dog	V
LV 674	L. sp.	L78	Rioux (14)	Tunisia	Dog	V
LV 675	L. donovani	B14	Bray (20)	Senegal	Dog	V
LV 676	L. hertigi	CM170	Zeledon (18)	Costa Rica	Coendou mexicana	
LV 677	L. sp.	Salem	Custodio (57)	Honduras	Man	CL
LV 678	L. donovani	B12	Bray (20)	Senegal	Dog	V
LV 679	L. tropica	Ackerman	Hendricks (53)	Asia	Man	CL
LV 680	L. sp.	173	Hendricks (53)	Iran	Man	CL
LV 681	L. sp.	193	Hendricks (53)	Iran	Man	CL
LV 682	L. sp.	204	Hendricks (53)	Iran	Man	CL
LV 683	L. sp.	214	Hendricks (53)	Iran	Man	CL
LV 684	L. donovani		Roche (5)	Sudan	Man	V
LV 685	L. donovani		Roche (5)	India	Man	V
LV 686	L. braziliensis	Leger	LSTM (16)	F. Guyana	Man	CL
LV 687			LSTM (16)	India	Dog	V
LV 688	L. donovani	R 35	Bettini (55)	Italy, Grosseto	R. rattus	
LV 689	L. donovani	R 55	Bettini (55)	Italy, Grosseto	R. rattus	
LV 690	L. donovani	R 053	Bettini (55)	Italy, Grosseto	R. rattus	
LV 691	L. tropica	Stoke	LSTM (16)	Pakistan	Man	CL
LV 692	L. braziliensis	Hopkins	Chang (61)	Venezuela	Man	CL
LV 693	L. donovani	Kasur	Ashford (27)	India, Calcutta	Man	PKDL
LV 694	L. donovani	Ghosh	Ashford (27)	India, Calcutta	Man	PKDL
LV 695	L. tropica	OSF	Ashford (27)	India, Delhi	Mun	CL

LV No.	Species	Isolate No.	Donor	Where Isolated	Source	Notes
LV 696	L. donovani	JCB	Ashford (27)	India, Bihar	Man	KA
LV 697	L. aethiopica	5.104	Ashford (27)	Ethiopia		
LV 698	L. donovani		Ashford (27)	India, Bihar	Man	KA
LV 699	L. donovani	Sumitra Devi	Ashford (27)	India, Bihar	Man	KA
LV 700	L. donovani	DY6	Ashford (27)	India, Bihar	Man	KA

WR	BN	LIV	L. infantum (=L. donovani) (LV9)		L. major (LV39)		L. m. ankazonensis (LV78)		Comments
			ED_{90}	PI	SD_{90}	PI	SD_{90}	PI	
214975 AB	BE 45137	1593	120 p.o.	0.4					Glucantime (as Sb)
181023	ZN 39806	1594	~13 p.o.	~3.6					4-methyl primaquine
211666 AB	BG 11417	1595	~9 p.o.	~5.2					8-aminoquinoline
182234	BE 17580	1596	<10 p.o.	>4.7					2-methyl primaquine
182234 AC	BE 10198	1289			135	6.1	*		2-methyl primaquine
142490	AX 23187	1100	>MTD(=100)		NA MTD		*		Mefloquine
142490	AX 23187	1100	>MTD(=100) p.o.						Mefloquine
	BH 73074	1642	Toxic at 30/100		Toxic at 100		>MTD (=10)		
225448 AG	BH 58522	1647	>30	<1.6	*		*		
005990 AD	BE 20185	1648	>30	<1.6	*		*		8-aminoquinoline
221527 AB	BG 48898	1649	<10	>4.7	*		*		" (most recent batch tested)
227495 AA	BG 56738	1650	<30	>1.6	*		*		"
219423 AA	ZN 58285	1651	<10	>4.7	*		*		"

ED_{90}/SD_{90} = mg/kg x 5 sc PI = Pentostam Index NA = not active MTD = maximum tolerated dose

* To be repeated - unsatisfactory control infections

TABLE 1A

WR	BN	LIV	L. infantum (=L. donovani) (LV9)		L. major (LV39)		L. m. amazonensis (LV78)		Comments
			ED_{90}	PI	SD_{90}	PI	SD_{90}	PI	
113618	AT 88437	1098			94	8.8	*		
135403	AX 26982	1099			205	4.0	*		quinazoline

ED_{90}/SD_{90} = mg/kg x 5 sc PI = Pentostam Index/ NA = not active MTD = maximum tolerated dose

* To be repeated - unsatisfactory control infections

TABLE 1B

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 2

WR 214975 AB BE 46137

Compound: LIV | 1893 GLUCANTIME

Route of administration: p.o.

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	5	1360	
30.0	5	1757	100 ± 1.4
100.0	5	1179	86.7 ± 7.4
300.0	5	356	26.2 ± 7.7

ED_{50} 190 (90 - 250) 63 (30 - 83) as Sb ED_{90} 360 (170 - 490) 120 (57 - 163) as Sb Pentostam Index 0.4

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 3

WR161023 ZN 39806

Compound: LW1594 4-methyl primaquine

Route of administration: p.o.

L. donovani (Strain LV9) Experiment No.: Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	5	1360	
10.0	5	466	34.3 ± 7.8
30.0	5	0	0
100.0	5	0	0

ED_{50} ~ 8.5 ED_{90} ~ 13.0 Pentostam Index ~ 3.6

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

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Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 4

WR 211666 AB BG 114413

Compound: LIV 1598

Route of administration: p.o.

L. donovani (Strain LV9) Dose (mg/kg)	No of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	5	1360	
10.0	5	101	7.4 ± 2.6
30.0	5	0	0
100.0	5	> LD_{100}	

ED_{50} ~ 5.0 ED_{90} ~ 9.0 Pentostam Index ~ 5.2

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

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Liverpool School of Tropical Medicine

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Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 5

WR182234 BE19580

Compound: LIV/1596 2-methyl primaquine

Route of administration: p.o.

L. donovani (Strain LV9)

Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	5	1360	
10.0	5	0	0
30.0	5	0	0
100.0	5	0	0

ED_{50} < 10 ED_{90} < 10 Pentostam Index >4.7

L. major (Strain LV39)

Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78)

Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

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Liverpool School of Tropical Medicine

Signed:

Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 6

WR 182234 AC BE 10198

Compound: LV/1289 Route of administration: S.C.

L. donovani (Strain LV9) Experiment No.: Date:

Dose (mg/kg)	No of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control			

ED_{50} ED_{90} Pentostam Index

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0	0	0.6	0.8	3.0	3.2	3.4			
50.0	0	0	1.6	2.2	3.6	3.2	3.4	100	0	100
100.0	0	0	0.2	0	1.0	2.0	2.8	14.3	85.7	54.6

SD_{50} 103 SD_{90} 136 Pentostam Index 6.1

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD_{50} SD_{90} Pentostam Index

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Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS TABLE 7A

WR 142490 Ax 2319?

Compound: LV/1100 Mefloquine Route of administration: sc

L. donovani (Strain LV9) Experiment No.: Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	5	820	
30.0	5	735	89.6 ± 8.3
60.0	5	791	96.5 ± 4.3
100.0	5	466	56.8 ± 15.6

ED_{50} ED_{90} > MTD Pentostam Index

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control	0	0	0	0	0	0.2	1.4			
30.0	0	0	0	0	0	0.8	1.6			100
70.0	0	0	0							
100.0	0	0	0	0	0	0.5	2.2			100

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS TABLE 7B

Compound: WR 142490 Ax 23187 LIV/1100 Mefloquine

Route of administration: po.

L. donovani (Strain LV9)

Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	5	820	
30.0	5	851	100 ± 1.6
60.0	5	894	100 ± 1.5
100.0	5	798	97.3 ± 6.1

ED_{50} > MTD ED_{90} > MTD Pentostam Index

L. major (Strain LV39)

Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78)

Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 8

BW73034

Compound: Lv/1642

Route of administration: sc

L. donovani (Strain LV9)

Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	10	1181	
30.0	5	-	> LD_{100}
100.0	5	-	> LD_{100}

ED_{50} ED_{90} Pentostam Index

L. major (Strain LV39)

Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										
100.0										> LD_{100}

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78)

Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0	0	0.2	0.2	1.4	1.4	1.6			
10.0	0	0.2	0	0.2	0.6	0.8	0.8	100	0	54.2

SD_{50} SD_{90} > MTD (=10) Pentostam Index

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

V

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 9

WR 225448 AG BM 88522

Compound: LV/1647

Route of administration: SC.

L. donovani (Strain LV9)

Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	10	772	
30.0	5	162	21.0 ± 19.2

ED_{50} < 30 ED_{90} > 30 Pentostam Index < 1.6

L. major (Strain LV39)

Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78)

Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS TABLE 10

WR 005990 AD BE 20185

Compound: Liv 1648 Route of administration: SC

L. donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	10	772	
30.0	5	489	63.4 ± 5.1

ED_{50} ED_{90} >30 Pentostam Index <1.6

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 11

WR 221627 AB BG48898

Compound: LIV/1649 Route of administration: s.c.

L. donovani (Strain LV9) Experiment No.: Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	10	772	
10.0	5	0	0

ED_{50} <10 ED_{90} <10 Pentostam Index >4.7

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE :2

Compound: WR 227495 AA BG58738 LIV | 1680

Route of administration: S.C.

L. donovani (Strain LV9)

Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	10	772	
30.0	5	0	0

ED_{50} <30 ED_{90} <30 Pentostam Index >1.6

L. major (Strain LV39)

Experiment No.:

Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78)

Experiment No.:

Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

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Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS TABLE 13

WR 219423 AA ZN 53285

Compound: LV 1661 Route of administration: S.c.

L. donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	10	772	
10·0	5	0	0

ED_{50} < 10 ED_{90} < 10 Pentostam Index >4·7

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

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Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS TABLE 14

WR 113618 AT 88437

Compound: LIV/1098 Route of administration: S.C.

L. donovani (Strain LV9) Experiment No.: Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control			

ED_{50} ED_{90} Pentostam Index

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control	0	0.2	1.2	2.2	3.4	4.0	4.0			
50.0	0	0	0	0.8	2.4	3.2	3.6	22.2	77.8	66.7
70.0	0	0	0.2	0.8	3.6	3.6	4.0	27.8	72.2	81.3
100.0 ($\sim LD_{50}$)	0	0	0	0	0	0	1.0	0	100	6.7

SD_{50} 68 SD_{90} 94 Pentostam Index 8.8

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

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Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

TABLE 15

WR 135403 Ax26982

Compound: LV/1099

Route of administration:

L. donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control			

ED_{50} ED_{90} Pentostam Index

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0	0	0.6	0.8	3.0	3.2	3.4			
150.0	0	0	0.6	2.0	3.4	3.0	2.4	100	0	100
200.0	0	0	0	0.2	0.8	1.0	2.2	14.3	85.7	38.2

SD_{50} 185 SD_{90} 205 Pentostam Index 4.0

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index

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Liverpool School of Tropical Medicine

Signed:
Date:

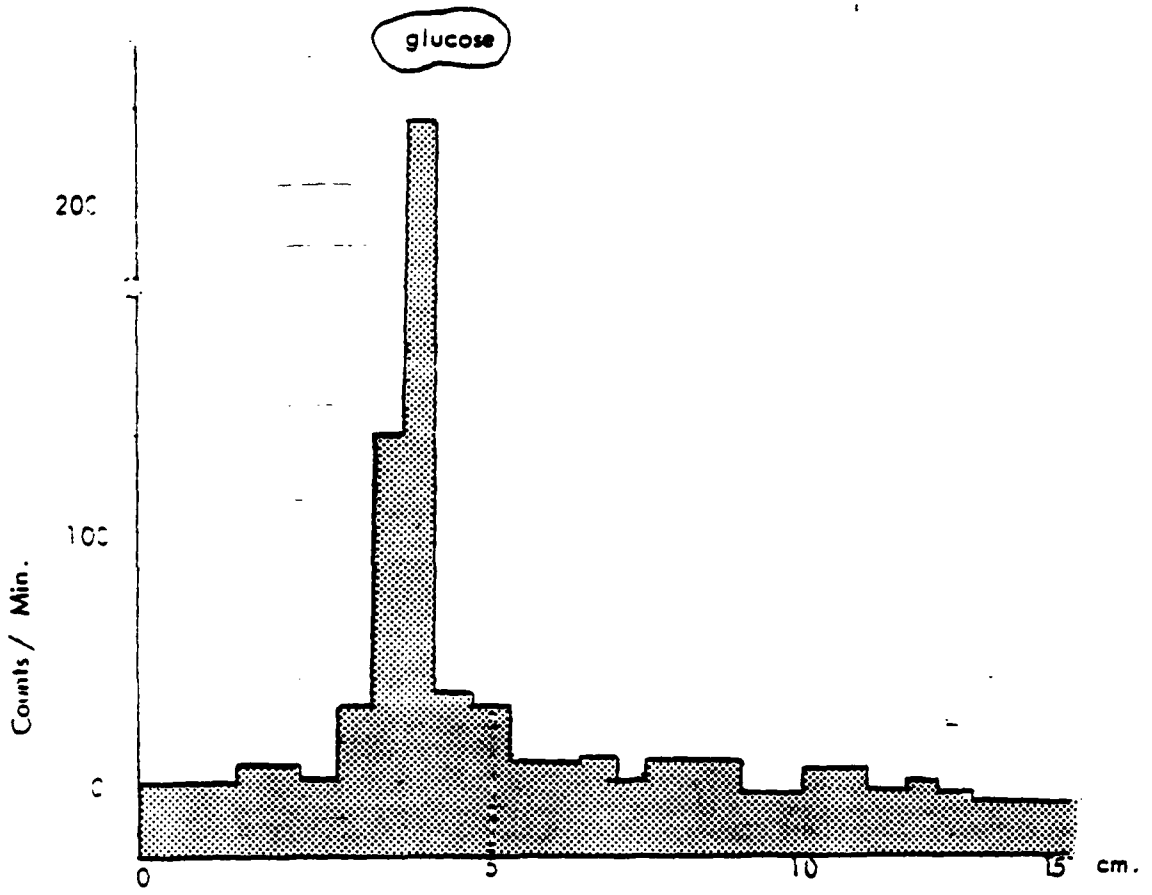
Principal Investigator: Professor W. Peters

TABLE 16

Activity of various compounds against *Leishmania* in tissue culture

DRUG	HOST	PARASITE LINE							
		L. donovani s.l. WR 130 (LV649)		*L. panamensis* WR 128 (LV648)		*L. major* LV39		*L. m. amazonensis* LV78	
		Activity	TI(MTD)	Activity	TI (MTD)	Activity	TI(MTD)	Activity	TI (MTD)
Amphotericin B	MPM	3	10(1)	3	10(1)	2	10(1)	3	100(1)
Nystatin	MPM	2	<1(100)	3	10(10)			3	10(100)
4-methyl primaquine WR 181023	MPM	2	1(10)	3	10(10)	3	10(100)	3	10(100)
2-methyl primaquine WR182234	MPM	2	<1(10)	3	>10(10)	3	10(100	2	10(100
WR 6026	MPM	1	<1(10)	0-1	<1(10)	1-2	<1(10)	1	<1(10)
	DS							3	>1(10)
WR 211666	MPM	1	<1(1)	1	<1(10	0-1	<1(1)	1	<1(10)
	DS							3	>1(10)
25-hydroxy cholesterol	MPM							0	-(>100)
Allopurinol	MPM	0	-(>1000)	0	-(>1000)	0	-(100)	0	-(>1000)
Oxypurinol	MPM	0	-(>100)	0	-(>100)			0	-(>1000)
BH 73074	MPM	0	-(<100)	0	-(<100)			0	-(0.1)

Dose = concentration μg/ml
Score = 0; 1-active at toxic dose only; 2-some action at non-toxic dose; 3-fully active at non-toxic dose.
TI = Therapeutic Index (at MTD)
MPM = mouse peritoneal macrophage
DS = dog sarcoma



Paper chromatography of final medium (ether extract) with [^{14}C] glucose after 1 hour incubation with amastigotes of L. m. amazonensis .

FIGURE 1

Metabolism of ^{14}C glucose by amastigotes of L. m. amazonensis in vitro

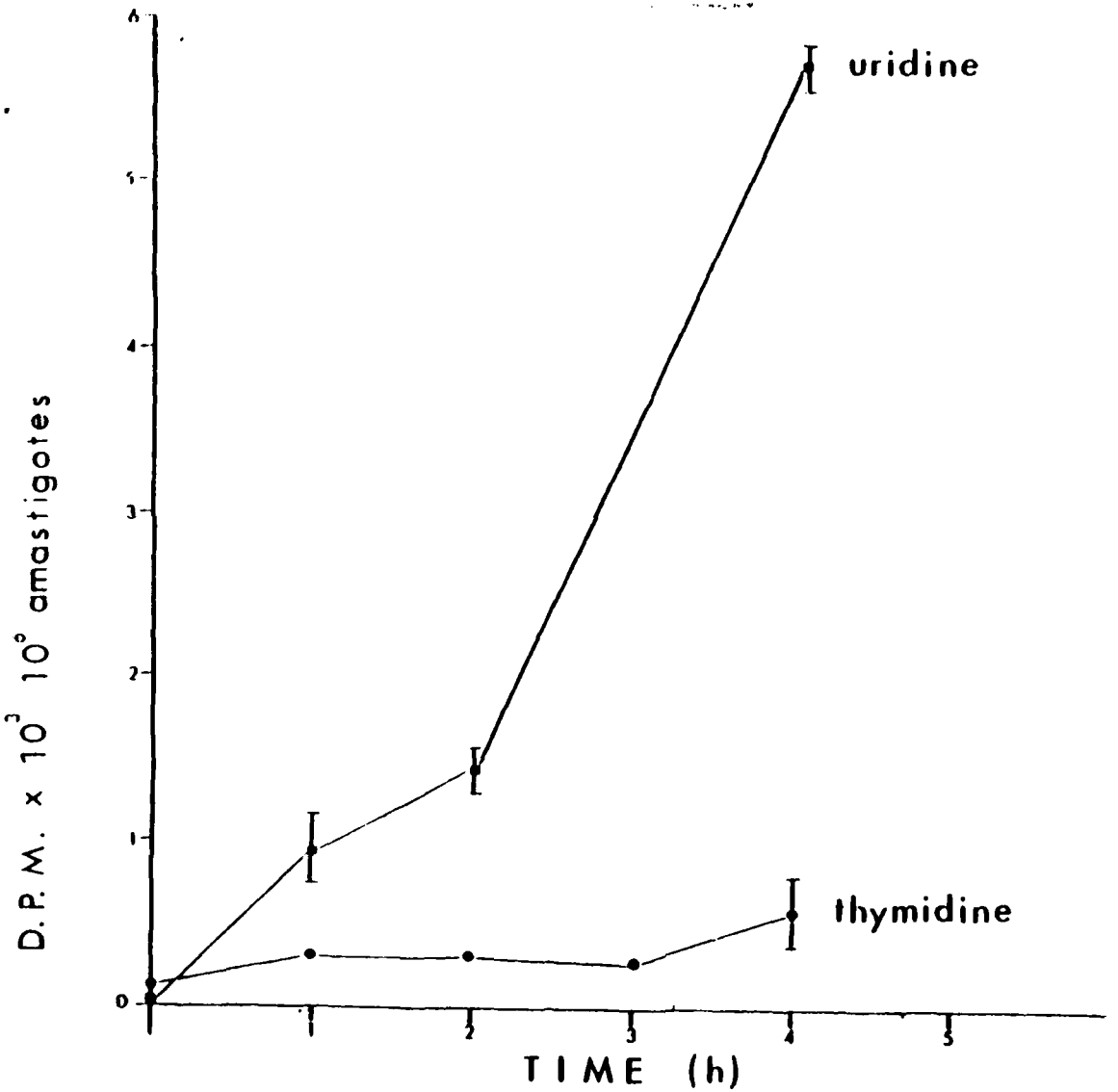


FIGURE 2

Incorporation of ^{3}H uridine and ^{3}H thymidine by amastigotes of L. m. amazonensis in vitro

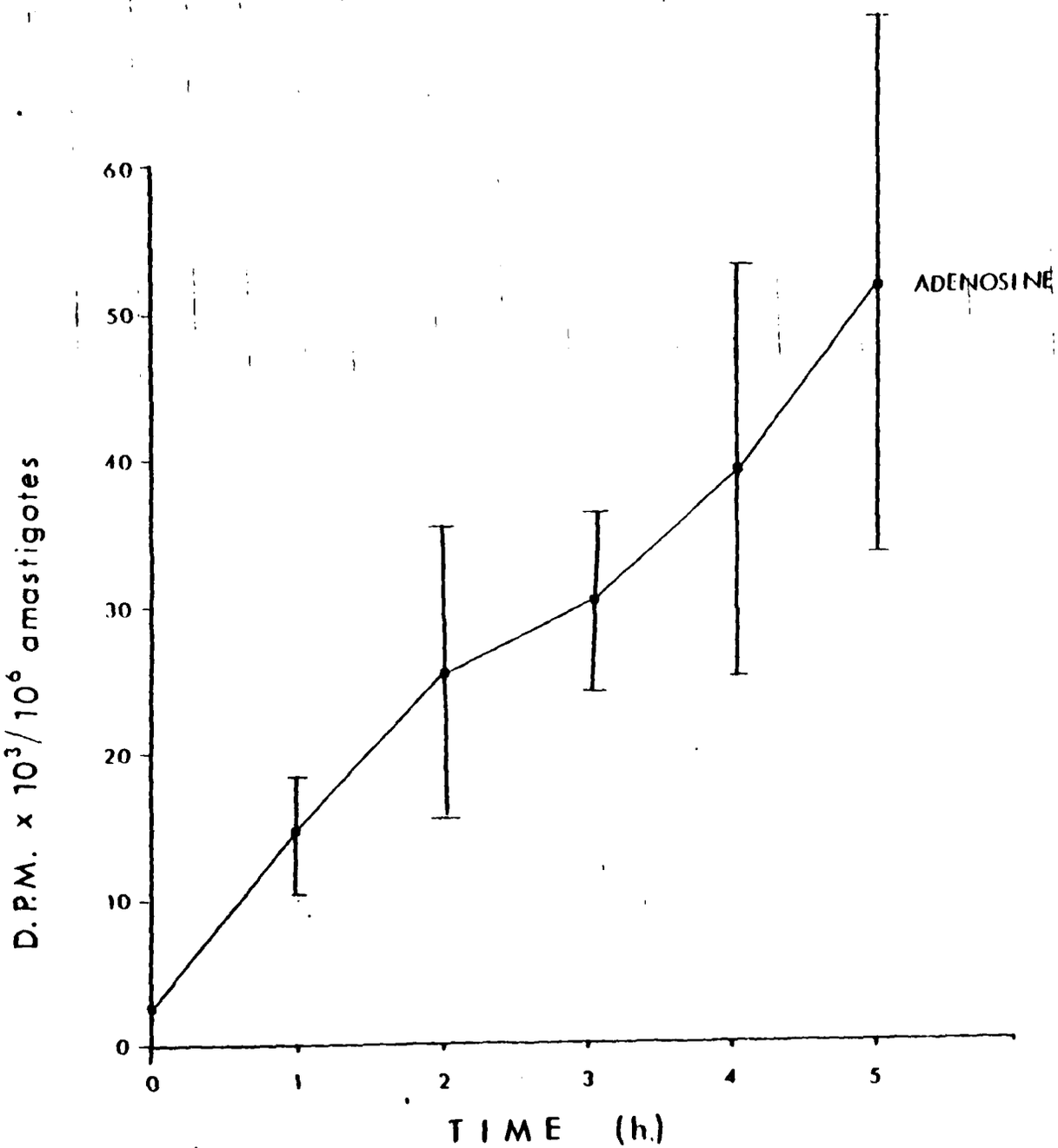


FIGURE 3

Incorporation of ^{3}H adenosine by amastigotes of L. m. amazonensis in vitro

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